DATA EVALUATION RECORD
BEE MONITORING DURING MAIZE FLOWERING IN THE RHINE VALLEY
(Non-guideline study)

1. CHEMICAL: Clothianidin PC Code No.: 044309

2. TEST MATERIAL: Clothianidin Technical Purity: Not reported

3. CITATION:

Authors:	G. Liebig, T. Kustermann, and D. de Craigher
Title:	Bee monitoring during the 2008 maize flowering season in the Rhine valley.
Study Completion Date:	June 31, 2008
Laboratory:	Regional Bee Research Insitute at Hohenheim University. Faculty of Agricultural Sciences (300) 70593 Hohenheim GERMANY
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4. REVEIWER BY: Christina deMariano, OPP/EFED/ERB5

Signature: **Date:**

5. APPROVED BY: Allen Vaughan, Entomologist, ERB-V

Signature: **Date:**

6. STUDY PARAMETERS:

Test species: Apis mellifera
Test duration: approximately 5 months

7. CONCLUSIONS:

Continual observation of bee colonies damaged by maize sowing and of new colonies fed on contaminated pollen combs did not reveal any lasting intoxication. A dilution effect due to the continued collection of pollen probably had a part to play.

The damaged colonies recovered over the course of the summer and did not suffer any further harm to their development during the maize flowering season. The monitoring colonies set up in the affected areas in June were not damaged during the maize flowering season either. Productive colonies, new colonies and sub-colonies set up without any pollen supplies shortly before maize came into flower did develop differently at the three Rhine valley sites, but colony development was not worse at any of these sites than at the control sites in the Stuttgart area, of which some were exposed to maize and some were not.

The foraging activity on maize to collect pollen by the colonies in the Rhine valley varied in intensity. The proportion of maize pollen in the samples taken from pollen traps, which were only used for short periods, was between 0 and 80%, with the average ratio being about 15%. Colonies did not use maize to top up their pollen stocks.

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: This is a non-guideline study.

C. Repairability: None

9. GUIDELINE DEVIATIONS: This is a non-guideline test.

10. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of TI-435 50 WDG to honeybees for the purpose of lifting the suspension on seed treatment for corn.

11. MATERIALS AND METHODS:

A. Test Organisms

Species: Apis mellifera

B. Test Design

Trial colonies and methods

In the last ten days of June 2008, eight productive colonies and four new colonies were taken to each of three sites with fields of maize grown from seed dressed with "Poncho Pro®" near Mullheim, Kippenheimweiler and Oberbruch. The Kippenheimweiler and Oberbruch sites were within flying distance of large areas of woodland, while the Mullheim site was not near any woodland.

The first population survey carried out on 4 July showed that each of the Rhine valley sites with four colonies had produced a sub-colony with a queen in the honey space. As these sub-colonies start off without pollen stocks they are ideal for assessing the quantity and quality of pollen available at a site. They have been used at Hohenheim for the past four years to assess "problem crops" (rapeseed, sunflower, and maize grown from dressed seed). This involves investigating the following parameters:

1. The size of the brood produced within three weeks (during the flowering season), measured by the number of bees in the sub-colony. This allows us to ascertain the suitability of freshly collected pollen for rearing the brood.
2. Fledged bee losses while the first brood is being produced or is emerging. This indicates harm or danger to bees that forage on the flowering crop.
3. Bee losses after the first brood has emerged. This demonstrates the quality of this brood that was reared while the plants were in flower.

The remaining populations that had been set aside when the sub-colony was formed ("brood populations") were captured four days later and used to rear queens, a process that started on 8 July at the beginning of the maize flowering season. The young queens developed normally. The queen-rearing colonies were split up on 21 July (after the queens had emerged). 36 breeding groups were created at each site, each with one "bee comb" occupied by 1,000 to 2,000 bees and one feed comb. They were placed in sets of four in four-frame hives and taken to Hohenheim where they mated (mating success rate 84%). Three further breeding groups were left at each site, made up of the "residual bees" of the queen-rearing colonies (bees on the inner walls of the hives and foraging bees outside the hive). These were much larger than the breeding groups that had been taken to Hohenheim. All nine queens started to lay eggs before the end of July. The development of the new colonies produced from these breeding groups has also been monitored

since 29 July. Their first brood was raised towards the end of the maize flowering season.

As part of the census carried out on 8 July, a dead bee trap (Münster model) was placed in front of each productive colony, new colony and sub-colony and pollen traps were placed in front of every three productive colonies, new colonies and sub-colonies. These were to be activated by the local "field troops" working with each colony and each group. However, it soon became evident that pollen was being found even in the pollen traps that were not completely sealed, though in smaller amounts. For that reason we decided only to activate the pollen traps (all of them) when we needed to handle the colonies. The pollen which had then been brought back within the past few hours was collected and taken back to the laboratory to be sorted into types (maize or non-maize).

Sub-colonies formed from productive colonies with queens in the honey space were also formed in July 2008 at three sites (plus six control sites) in the Stuttgart area (at Nurtingen, Kirchheim/Teck and Rottenburg), and their development was monitored. The Rottenburg colonies had four hive locations and three woodland locations (hut, fir tree and crate. Bees at the last three sites only come into contact with intensively farmed land at the very end of their flight range (> 1.5 km). The crops available in a closer flight range (< 1 km) were maize and sunflower in the case of Nurtingen and maize and asparagus in the case of Kirchheim. The development of 12 new colonies at an apiary near Deizisau was observed. Three of these new colonies were given two pollen combs each in June that had been taken from the six damaged productive colonies belonging to beekeeper S. The bee bread was contaminated with clothianidin at an average rate of 7 ppb. Three new colonies were each given one pollen comb from the productive colonies of beekeeper L. The bee bread contained 34 ppb of clothianidin. The chemical analyses were conducted by the Speyer Agricultural Research Institute. The 12 new colonies of an apiary near Balzholz were used in an exchange trial in which, after the maize fields near Mahlberg had been treated with Biscaya in July, six uncontaminated new colonies from Balzholz were exchanged with six new colonies belonging to beekeeper L which might have been contaminated. We were therefore able to closely monitor 12 new colonies on both of these sites.

The development of twelve productive colonies at each of the following sites (Aalkistensee near Olbronn, Schlatt, Rottweil and Onolzheim) was monitored from March 2008. The trial colonies in Onolzheim are directly opposite a five-hectare field of maize grown from seed dressed with "Poncho Pro®". Another smaller field is less than 100 metres away. The maize was sown in early May and came into flower about three weeks later than that grown in the Rhine valley, which is warmer. Pollen traps were placed in front of the hives used by all the colonies during the maize flowering season, but were only emptied twice. In both fields stalks were cut and the pollen was collected by shaking them when the maize first came into flower. All the colonies were observed by means of censuses carried out according to the Liebefeld method. They were generally conducted every 21 days. The censuses were conducted more frequently during the maize flowering season at the Müllheim, Kippenheimweiler and Oberbruch sites in the Rhine valley. Bee samples were taken during the censuses, and used mainly to investigate for disease (nosema, amoebic dysentery, varroa). Most of these investigations require the use of a microscope. They are

very time-consuming in the field and for this reason, along with the large number of samples collected, they cannot be carried out soon after the samples are taken. Priority was given to the bee samples (S and L colonies) collected in the Rhine valley and the monitoring colonies at the Müllheim, Kippenheimweiler and Oberbruch sites.

Waste samples from all the trial colonies were diagnosed at regular intervals starting in August in order to determine the natural level of varroa mite infestation and varroa mortality following treatment. Varroa treatments were conducted using formic acid or oxalic acid, following the Baden-Wurttemberg varroa control recommendations or the alternative "divide and treat" system.

12. REPORTED RESULTS:

The development of bee colonies damaged by maize sowing

The first census conducted on 15 May found that the L colonies near Mahlberg, which had clearly suffered more damage and whose bee bread was around five times more heavily contaminated with clothianidin, were much weaker than the S colonies near Iffezheim, but that they produced relatively larger brood. Some of the L colonies still had clear brood damage, but this was not observed in the following censuses. All the censuses apart from the second one carried out on *5* June showed that on average the L colonies always produced much smaller broods than the S colonies, and that therefore the population numbers were always lower. The constant and more dramatic reduction in brood size during the summer among the L colonies, which were taken to forage on fir trees near Zell a.H. for a time in June and July, could be attributable to the much lower amounts of pollen available. This possible correlation between pollen supplies and brood performance is also seen at the three monitoring sites (Müllheim, Kippenheimweiler and Oberbruch). The Kippenheimweiler apiary had the least pollen available to it and performed worse in terms of brood production among the new colonies and sub-colonies (which started with very little pollen).

Factors such as the brood behaviour of the new colonies in Deizisau contradict the idea of a clear link between pollen supplies and brood performance. These smaller colonies, which had access to just as little pollen, raised much larger broods than the L colonies in July and August. It is likely that the amount of pollen actually consumed by a bee colony in summer is many times higher than the amount of pollen it has in store. We assume that while a brood is being reared, the amount of pollen or pollen cells consumed is roughly equivalent to the size of the brood or the number of brood cells being raised. Even the S colonies, which had plentiful supplies of pollen, consumed about twice as much pollen in summer than they had in store. The development of the S and L colonies from June onwards can be regarded as normal compared to the control and monitoring sites

Development of new colonies provided with contaminated pollen combs

The new colonies that received contaminated pollen combs were on average the same size as the

control colonies (about 3,000 bees), but produced slightly smaller broods. The introduction of contaminated pollen traps immediately after the first census had no effect at all, probably because of a dilution effect caused by freshly collected pollen. The colonies of both groups expanded their brood nest continuously until the start of August. The average maximum brood size on 1 August was 23,000 cells, with an average colony size of 10,000 bees. Average pollen stocks were always below 5,000 cells. The brood nest in both colony groups shrank dramatically in August and September, but this is normal.

Pollen stocks in combs during the maize flowering season

There was considerable variation in pollen stocks during the maize flowering season in July, particularly among the productive colonies. Over the course of the month, the number of cells at the Müllheim sites rose from an average of just under 8,000 to over 13,000, while levels in Oberbruch remained more or less the same and fell sharply in Kippenheimweiler from an average of around 6,000 cells to 2,000 cells.

These differences between the sites were also observed among the new colonies. The new colonies in Müllheim consistently had the best pollen supplies, while those in Kippenheimweiler always had the worst. Initially the pollen supplies available to the new colonies in Oberbruch were only slightly better than in Kippenheimweiler, but from mid-July pollen stocks started to increase dramatically, reaching the same level as in Müllheim by the end of the month.

The sub-colonies had been set up on 4 July with no pollen stocks. By the middle of July pollen stocks peaked for the first time at all sites at an average of approximately 2,500 cells. This was despite the fact that the sub-colonies in Müllheim initially collected more pollen than those in Kippenheimweiler and Oberbruch. Pollen stocks declined dramatically in Müllheim and Kippenheimweiler in August (while the maize crop was still in flower), and only the sub-colonies in Oberbruch were able to keep pollen stocks constant.

Overall, the change in pollen stock levels in July and August among productive colonies, new colonies and sub-colonies seems to indicate that pollen supplies in July and August were always lower in Kippenheimweiler. Pollen was most abundant in Müllheim during the maize flowering season and in July, and in Oberbruch after the maize flowering season and in August. This may be because the colonies in Oberbruch appeared to forage more intensively on maize possibly because the weather conditions in Oberbruch are wetter and because Himalayan balsam, a plant that supplies both pollen and nectar, was in flower in Oberbruch from the end of July.

Proportions of maize pollen in the pollen traps

The highest proportion of maize pollen was collected by the colonies in Oberbruch (average proportion in the pollen traps 22%), with the lowest proportion being found in Müllheim (average proportion 6%). However, there were marked differences between the individual colonies. At each site there were some colonies that collected no maize pollen and some in whose pollen traps relatively high proportions (up to 80%) of maize pollen were regularly found. These differences

are not correlated with the variation of pollen stocks in the combs or with colony development.

13. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors. Damage colonies recovered over the course of the summer. Bees monitored were not assessed after a "over wintering" period. This could have provided better data endpoints then assessing damage to the hive during a single season.

Colonies in the Mahlberg region were highly contaminated with clothiandin and were much weaker when compared to colonies in the Iffezheim region. Mahlberg bee bread contained five times the amount of clothiandin than bee bread in Iffezheim.

Development of colonies with contaminated pollen combs was normal and attributed to dilution of clothiandin from new untreated pollen being foraged.

Maize collected by colonies varied greatly even if the colonies at the same location. Maize was in total pollen loads from 0 to 80%. Regardless of amount, all colonies were highly producing.

Clothiandin was found in maize pollen at 0.3 to 11.4 µg/kg and it metabolites were below levels of detection of 0.3 µg/kg.

Bee bread from two colonies was contaminated with clothianidin 7 and 34 ppb. This study did recover high clothiandin in bee bread and pollen but is classified as supplemental and does not satisfy any EPA guideline.